Aerated Stratification Improves Germination of Ocala Sand Pine Seed

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A stratification system of soaking in water with and without aeration followed by moist chilling was tested for its effect on the germination of Ocala sand pine (Pinus clausa *var.* clausa *D. B. Ward*) *seed. The most beneficial treatment was a 24-hour aerated water soak at 25 °C followed by draining and moist cold storage at 4 °C for 21 days, and then surface drying after 21 days for either 1 week or 6 months. Seed receiving this treatment germinated faster than untreated control seed that received no moist conditioning or surface drying. The germination value, a combined measure based on both speed and completeness of germination, was more than double that for control seed. In addition, the 6-month storage at high moisture levels did not reduce germination percent. Thus, managers could treat seed prior to the sowing season and keep them in cold storage for use as needed. Although commercial nurseries commonly use stratification for sand pine, none of those contacted used aeration during the soaking phase. Stratification with a non-aerated soak can actually reduce germination percent.* Tree Planters' Notes 42(1):22-26; 1991.

Stratification—chilling under moist conditions to remove seed dormancy—has long been recognized as a useful method of treating seed to improve speed and amount of germination. Heydecker and Coolbear (*9*) reviewed many other presowing treatments that increase the speed and completeness of seed germination. Because Ocala sand pine seed is not dormant (*4*), it is often assumed that preconditioning treatments are unnecessary. However, Barnett (*3*) found that aerated water soaks would promote the germination of other nondormant southern pine seed. Previous work indicated that some type of presowing treatment (*10*), although not necessary to obtain good germination, might increase the germination rate of Ocala sand pine seed.

The primary objective of this study was to test the effect of different presowing treatments on the germination of Ocala sand pine seed. A secondary objective was to determine if seed, given a stratification treatment, could be stored for up to 6 months with comparatively high moisture content without a reduction in percent germination.

Materials and Methods

All seed used in the study were from seedlots used for regeneration on the Ocala National Forest, located in Florida. Cones that had matured in 1985 were collected in the fall of that year. These cones were gathered from many trees on fall timber sale areas at various locations across the Ocala National Forest.

After collection, cones were shipped to the USDA Forest Service, Ashe Nursery near Brookl MS. They were opened by dipp for 5 to 10 seconds in boiling water followed by kiln drying a 45 °C for 24 to 48 hours. After seed were extracted from open cones by shaking, they were dewinged and cleaned and ther shipped back to the Ocala Nati al Forest. Seed were kept in col storage there for about 3 month until they were obtained in Janu 1986 to conduct this study.

Equal amounts of four seedlot were mixed and used for the firs portion of the study. An additio seedlot from the 1986 collectio season was included in the sec phase of the study described be because only two of the origina 1985 seedlots had enough seed remaining for the test.

In the first phase of the study, three presowing treatments with three replications per treatment 300 seed per replication were applied to the bulked seedlot.

Treatment one, **non-aerated soak**, consisted of soaking seed distilled water at 25 °C with con stant light for 24 hours, draining excess water, and storing seed f 21 days at 4 °C in plastic cups with snap caps.

Treatment two, **aerated soak**, was the same except that the soa ing was done in water aerated b bubbling compressed air into the bottom of the container at a rate 6 liters/minute for each liter of

water. This caused the water to mix in a manner comparable to water at a moderate boil. The amount of water used for soaking treatments one and two was equal to the level of the 300 seed when put into a graduated cylinder; that is, if the seed filled the container up to 15 ml, then 15 ml of water was used.

Seed in treatment three, the **control,** were kept dry throughout the treatment period but were exposed to the same temperature and light conditions as the other treatments.

Following the 21 days of cold moist storage, the seed from all three treatments were surface-dried at room temperature. After surface drying, a subsample of 50 seed was removed from each replication. The remaining seed were placed back in cold storage for an additional 6 months. To obtain percent moisture content, seed was removed in subsamples, dried for 24 hours at 105 °C, then reweighed. After 6 months of additional cold storage, a second subsample of 50 seed was drawn from each replication, and fresh and dry weights were obtained as explained above. A third subsample of 100 seed, also drawn after the 6 months of additional storage, was taken from each replication of each treatment and placed on absorbent paper in germination boxes. After the paper was wetted with distilled water, boxes were placed in a germination room at 25 °C with constant light. The number of normal germinants as defined by the Association of Official Seed Analysts (*2*) was recorded daily for 21 days.

The second phase of the study used the same three treatments described above. However, seed were kept in cold storage for only 1 week instead of 6 months after surface drying. Treatments were applied to three replications, with 300 seed per replication, of three different seedlots. After the week in cold storage, seed were sown in germination boxes and monitored, as explained above.

Germination percent, germination rate, and germination value (*7*) were calculated to evaluate treatments. Germination percent, the percentage of seed which germinated before the end of the study, measures completeness of germination. Germination rate, the number of days required for 50% of the sown seed to germinate, is an index of the speed of germination. Germination value, the mean daily germination multiplied by the highest daily germination, combines both speed and completeness of germination. High values are desirable for both germination percent and germination value, but for germination rate, more rapid germination is shown by smaller values. Treatment means for all measures were compared by analysis of variance after arc-sine transformation of percentages to correct for non-normality.

Results

The first subsamples of seed removed had mean dry weight moisture contents after surface drying of 10, 35, and 32% of those for seed receiving the control, nonaerated soak, and aerated soak treatments, respectively. The second subsamples, which were drawn after surface drying and then 6 months of additional cold storage, had moisture contents of 7, 24, and 22% for the same treatments. This loss of moisture occurred because the containers used for seed storage were not airtight and the humidity was low inside the frost-free refrigerator where containers were kept.

The non-aerated soaking treatment followed by 6 months of cold storage significantly reduced germination percent, whereas the aerated soak treatment increased it slightly but significantly, compared with untreated control seed (table 1). Seed given the aerated soak treatment reached 70% germination after just 4 days at 25 °C, whereas control seed had not begun to germinate. The germination profile (figure 1) shows seed given the aerated soak treatment reached peak germination after 3 days. Germination of control seed did not peak until day 10. The non-aerated soak treatment caused seed to begin germination sooner than the control seed. However, for these two treatments it took about the same number of

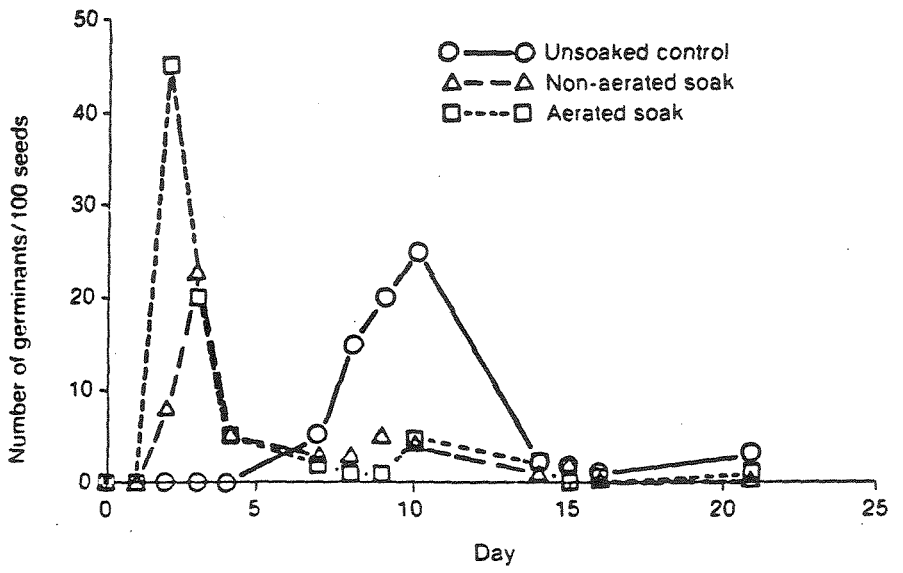

Figure 1—*Effect of different presowing treatments on the daily germination of Ocala sand pine seed after 6 months in storage. Data points are means of three replications.*

days for 50% of the seed in each treatment to germinate (table 1). Soaking seed without aeration did not improve the germination value. The germination value for seed given the aerated soak treatment however, was nearly three times greater than the control value.

In the second part of the study with 1-week storage, there were no significant differences in germination percent, germination rate, or germination value due to seedlots. Because there was no seedlot effect, data were pooled and only treatment means are presented (table 1). Although the absolute values were different, seed stored for 1 week responded to the aerated soak treatment much like seed stored 6 months. The aerated soak treatment increased the germination rate compared to untreated control seed (3.7 days vs. 8.4 for controls). In addition, there was an increase in germination value over the control. As before, soaking without aeration reduced germination percent and did not improve germination rate or value relative to control seed.

Discussion

Physiologically, germination can be divided into three phases (6): reactivation of pre-existing metabolic systems, synthesis of enzymes for degradation of reserves, and finally, synthesis of new cellular components. Alvarado and Bradford (1) and Savino et al. (11) have suggested that presowing treatments can activate the metabolic processes necessary for germination. The most probable explanation for the increased germination rate of seed that had received the aerated soak treatment is that some of these metabolic processes had already taken place during the soaking and 21-day moist cold-storage phases of seed treatment. Thus, stratification can increase the germination rate because it gives

Table 1—*Germination percent, germination rate (days to 50% germination), and germination value (7) of Ocala sand pine seed after different presowing treatments and storage times*

Seed treatment	Germination (%)	Germination rate*	Germination value
6 months of storage			
Control	75 b	10.0 b	23.2 a
Non-aerated soak	56 a	9.7 b	27.8 a
Aerated soak	81 c	2.7 a	86.9 b
1 week of storage			
Control	82 b	8.4 b	7.2 a
Non-aerated soak	51 a	15.2 c	4.0 a
Aerated soak	88 b	3.7 a	18.8 b

Means in a column for each storage period not followed by the same letter are significantly different (LSD) at the .05 level. For 6 months, storage, means are for three replications of a mixed seedlot. For 1 week's storage, means are for 9 numbers, that is, three replications of three seedlots each.

*This is an inverse variable, where a lower absolute value means a faster germination rate.

seed time for metabolic activity before sowing.

Aeration applied during seed soaking treatments helps maintain adequate oxygen levels. Non-aerated soaks can be detrimental due to damage resulting from the development of anaerobic conditions as metabolic processes occur within the seed (*13*). This damage is the likely cause of the decline in germination percent, compared to untreated seed, for seed given the non-aerated soak treatment. Thus, for Ocala sand pine the non-aerated soak treatment should not be used because it reduces rather than increases overall seed performance.

The seed were stored for 6 months following treatment to see if there was a loss of germination percent from extended storage at comparatively high moisture levels. Past work had shown sand pine seed could be stored for up to 10 years at moisture contents of 15% and a temperature of 1 °C (*5*). This study shows that Ocala sand pine seed can be stored for 6 months at a moisture content of 20 to 30% and 4 °C without reducing germination percent if the moisture content is initially increased by a 24-hour aerated soak treatment, followed by 21 days in cold storage and then surface drying.

Seed treated by this method germinated faster and more completely than control seed after either 1 week or 6 months of storage. Thus, it would be possible for managers to treat large seed quantities and store them for use over the sowing season. Some fungal growth was noted on aerated soaked seed after 6 months of storage. However, this did not effect germination percent, rate, or value. Fungal growth could be reduced by washing seed with 5% sodium hypochlorite solution before soaking (*8*).

Stratification using a 24-hour soak treatment followed by a 3- to 6-week period of cold, moist storage is a routine method of breaking dormancy of southern pine seed. It is also a common method of breaking dormancy of conifer seed in the West, where it is called naked stratification (*12*). A personal survey of industrial and State nurseries in Florida revealed two that grow Ocala sand pine. Both use stratification, but neither apply aeration during the soaking phase. Use of aeration should benefit these operations since, as shown by this study and discussed above, non-aerated soaks can reduce germination.

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